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Ketamine Alters Rat Flash Evoked Potentials[1]

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RIGDON, G. C. AND R. S. DYER. *Ketamine alters rat flash evoked potentials.* PHARMACOL BIOCHEM BEHAV 30(2) 421–426, 1988.—Discovering the neurotransmitters involved in the generation of flash evoked potentials (FEPs) would enhance the use of FEPs in screening for and assessment of neurological damage. Recent evidence suggests that the excitatory amino acids, glutamate and aspartate, may be transmitters in the visual system. Ketamine selectively antagonizes the actions of excitatory amino acids on n-methyl-d-aspartate receptors and may be administered systemically. Two experiments were designed to test the effects of ketamine on rat FEPs. First, the effects of ketamine (37, 75, 150 mg/kg) on FEPs recorded in light and dark backgrounds were investigated at a single (10 min) posttreatment interval. Ketamine administration resulted in dose-dependent alterations in FEP peak amplitudes and latencies. Peak P1 amplitude increased by a factor of 4, in a dose-dependent manner. Peak N1 virtually disappeared at 150 mg/kg. Peak P2 amplitude increased by 50%, but only in the light background, and only at 150 mg/kg. Second, ketamine (150 mg/kg) effects on FEPs were investigated 5 min and 30 min following administration. The decrease in peak N1 amplitude was maximal 5 min after administration and the amplitude was recovering at 30 min. The effects on peak P1 and peak N3 amplitudes were maximal 5 min after ketamine administration, but were not recovering 30 min postinjection. The various peak latencies were also affected differently. The possible role of glutamate or aspartate in the generation of rat FEPs is discussed.

Flash evoked potentials Ketamine

FLASH evoked potentials (FEPs) primarily reflect summed, neuronal, graded postsynaptic potentials. FEPs are used in neurological [26] and toxicological [9] practice as indicators of the functional integrity of the visual system. Relatively little is known about the neurochemical generators of FEPs. Discovering the neurotransmitters involved in the generation of FEPs would enhance their use in screening for and assessment of neurological damage.

Recent studies have indicated that either glutamate or aspartate (postulated neurotransmitters) may be released from retino-geniculate and cortico-geniculate fibers in the visual system [4–6, 13, 14, 21, 31]. If so, antagonism of the receptors which these excitatory amino acids act upon should produce alterations in FEPs. The "dissociative" anesthetic, ketamine, has been reported to cause transient blindness in humans during recovery from anesthesia [12]. Ketamine is a potent, selective, noncompetitive, antagonist of the actions of excitatory amino acids on n-methyl-d-aspartate (NMDA) receptors [1, 15, 20, 22, 28, 30], a class of receptors which mediates a prolonged depolarization associated with changes in membrane conductance. The ketamine blockade of NMDA-mediated effects is more potent than its antagonism of cholinergic receptors [22]. We investigated the effects of 0, 37, 75, or 150 mg/kg IP ketamine HCl on FEPs. Pilot data suggested that the effects of ketamine might depend upon background illumination, therefore, we recorded from rats under conditions of either light or dark background illumination.

METHOD

Adult, male Long-Evans hooded rats were used in this study. Animals were surgically implanted with recording electrodes under pentobarbital (50 mg/kg, IP) anesthesia. Recording and ground electrodes were 00-90 stainless steel screws threaded into burr holes drilled in the skull. Ground and reference electrodes were placed 5 mm anterior to and 2 mm left and right of bregma. Visual cortex electrodes were placed 6 mm posterior to bregma and 4 mm lateral to midline. Nichrome wires connected the screws to a plastic Amphenol connector. The assembly was covered with dental acrylic and the wound sutured shut. Each rat was given 100,000 units of penicillin G IM and returned to its cage. Animals were allowed a one week recovery period before testing.

[1]The research described in this manuscript has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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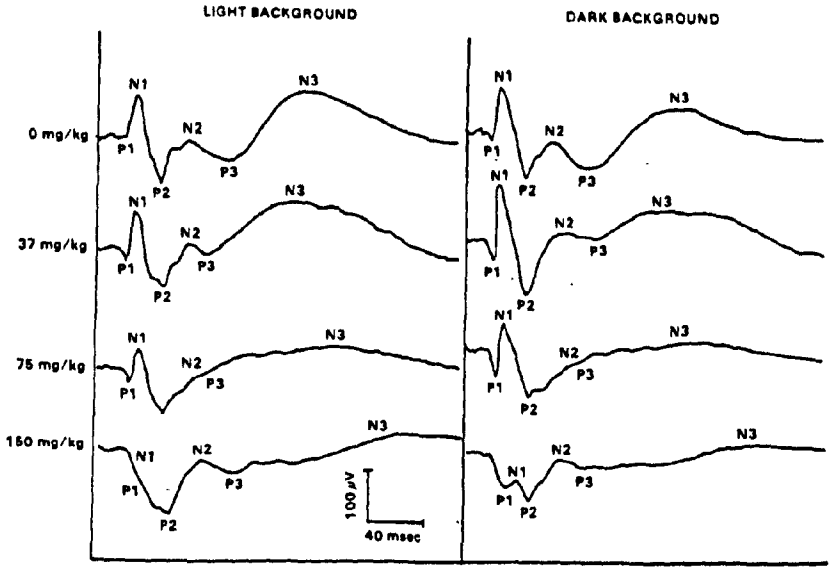

FIG. 1. Group average waveforms of FEPs from all rats in each dosage group in light or dark background 10 min after IP ketamine administration. The flash occurred at the beginning of each trace.

Animals were tested in rectangular boxes with mirrors on three sides, and a Grass PS-22 strobe on the fourth. High intensity (4.53×10^{-2} lux-sec) 10 μsec flashes were used as the eliciting stimuli. Animals were tested in either dark or light (115 lux) chambers following a 10 min adaptation period. Trials were presented at 0.3 Hz and 128 trials were averaged for each FEP waveform. To reduce the probability that ketamine would produce hypothermia, rats were maintained at a warmer ambient temperature (approximately 28°C) after treatment.

The sampling rate was 2500 Hz and the EEG was bandpass filtered between 0.8 Hz and 1 kHz. Averaging was accomplished by a PDP 11/70 computer. Peak latencies and peak amplitudes were obtained from each animal's waveform. Baseline was defined as the average voltage between 10 and 15 msec after the flash; a segment of the waveform which is prior to any major stimulus-induced deflections.

Experiment One: Dosage Effects of Ketamine

Animals (n=38) were administered isotonic saline (control), 37, 75, or 150 mg/kg ketamine HCl IP and then placed in the recording chambers for 10 min prior to testing. Rectal temperatures were obtained immediately after testing. Each animal was tested at each dosage (at least 48 hours between testing) and order of dosage was assigned randomly. Different groups of animals were tested under light (n=17) and dark (n=21) adapted conditions.

Experiment Two: Time Course of Ketamine Effects

Animals were administered either isotonic saline (n=19) or 150 mg/kg ketamine (n=19) IP and then FEPs were obtained in dark chambers at 0, 5, and 30 min postinjection. To evaluate the effects of ketamine on body temperature at a time beyond the 10 min period evaluated in the first study, separate groups of rats were tested, and administered either isotonic saline (n=6) or 150 mg/kg ketamine (n=6). The rats were retested 15 min, 30 min, and 60 min later. Rectal temperatures were recorded following the 60 min test. Only the temperature data from this group of 12 rats will be reported here.

Multivariate analysis of variance (MANOVA, SAS Institute) was used to analyze the data according to statistical design principles outlined by Muller *et al.* [24]. Only data which met these criteria are reported as significant. For Experiment One, the effects of dosage (within subjects), background (between subjects), and dosage × background interactions on peak latencies and peak amplitudes were analyzed. For Experiment Two, the effects of dosage (between subjects), time (within subjects) and dosage × time interactions were analyzed. Bonferroni correction procedures were used to maintain an overall alpha for the experiment of 0.05, and Geisser-Greenhouse corrections were applied in cases of repeated measures. As there were 6 amplitudes and 6 latencies measured in each experiment, with a MANOVA performed upon each, the adjusted alpha was 0.05/12=0.0042. Unless otherwise indicated all effects discussed in the results section achieved this level of significance.

RESULTS

While quantitative assessment of the behavioral correlates of ketamine was not attempted, qualitative observations were consistent with observations reported by others [25]. At the 150 mg/kg dosage rats appeared anesthetized. At 75 mg/kg their behavior would generally be described as cataleptic immobility, while at 37 mg/kg behavior ranged from mildly affected (stereotypic behavior) to ataxic.

Experiment One: Dosage Effects of Ketamine

The amplitudes of peaks P1, N1, P3, and N3 were all altered 10 min following treatment with ketamine (see group average waveforms in Fig. 1), independent of background illumination. Peak P1 amplitude was increased in a dose-dependent manner (Fig. 2). The effect was significant at all

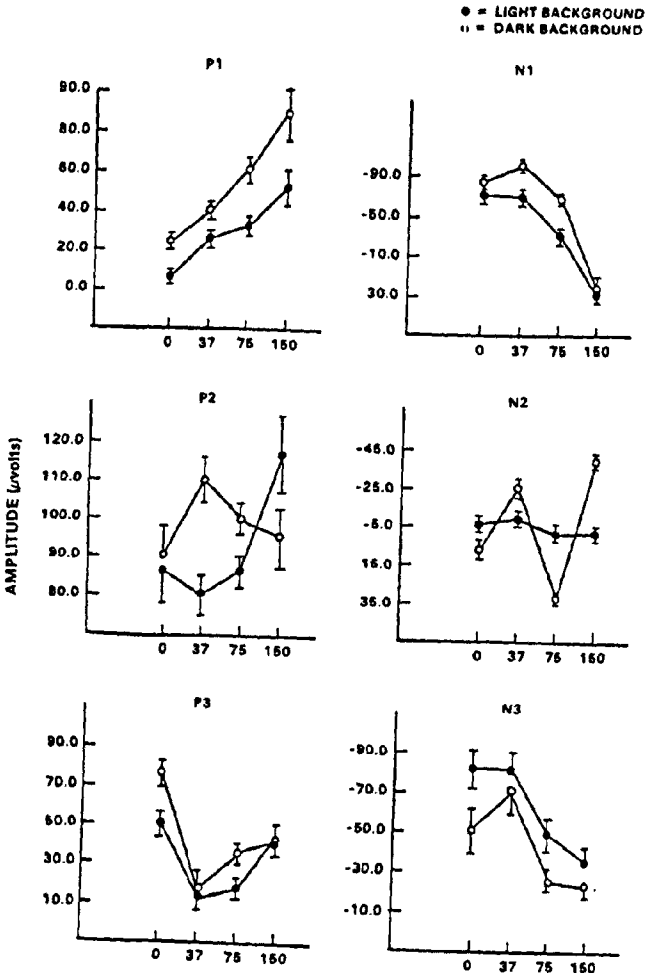

FIG. 2. Mean (±sem) for FEP peak amplitudes obtained in light or dark background 10 min after treatment with ketamine.

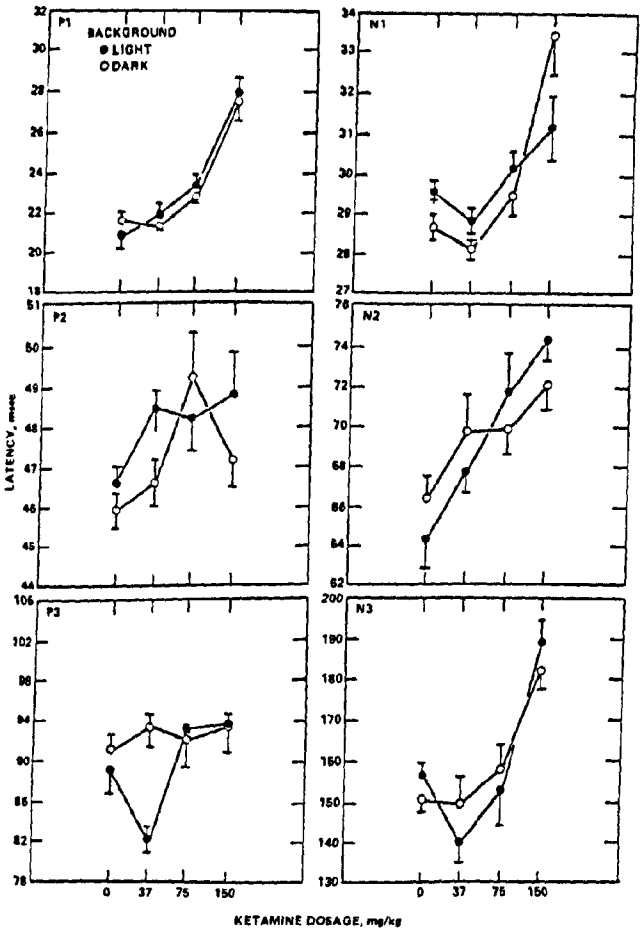

FIG. 3. Mean (±sem) for FEP peak latencies obtained in light or dark background 10 min after treatment with ketamine.

TABLE 1
EFFECTS OF KETAMINE ON RECTAL TEMPERATURE °C

	0 mg/kg	37 mg/kg	75 mg/kg	150 mg/kg
Dark-tested	38.4±0.07	38.7±0.08	38.4±0.16	37.8±0.12
Light-tested	38.2±0.10	38.8±0.10	38.3±0.11	38.0±0.10

Values are means±sem.

dosages. The mean peak P1 amplitude of animals treated with 150 mg/kg ketamine was 4 times the mean peak P1 amplitude of saline-treated animals. Peak N1 amplitude was decreased with increasing dosages of ketamine. While this effect appeared more pronounced in light-tested animals (Fig. 1), the absence of a significant dose × background illumination interaction failed to support this appearance. The decreased N1 amplitude was significant at the 75 and 150 mg/kg dosage levels, and in fact, peak N1 was virtually abolished in animals administered 150 mg/kg ketamine (Fig. 1). The amplitude of peak N3 was also decreased significantly following treatment with 75 or 150 mg/kg ketamine (Fig. 2). Peak P3 amplitudes were decreased by ketamine at the two lower doses, 37 and 75 mg/kg, but not at the 150 mg/kg dosage (Fig. 2).

Some of the effects of ketamine on FEP amplitudes were dependent upon background illumination. In light-tested rats peak P2 amplitude was increased by nearly 50% at the 150 mg/kg dosage, while, dark-tested rats had increased P2 amplitudes only at 37 mg/kg. In light-tested rats peak N2 amplitude was unaffected by ketamine, while in dark-tested rats the N2 peak amplitude was increased at 37 and 150 mg/kg, but decreased at 75 mg/kg.

The latencies of peaks P1, N1, N2, and N3 were significantly increased in a dose-dependent manner (Fig. 3). Peak P2 latency was increased following the lowest dosage. All peak latencies, except P2 and P3, were increased at the two higher dosages.

Animals became slightly (0.3 to 0.6°C) hyperthermic following the 37 mg/kg dosage, and slightly (0.2–0.6°) hypothermic following the 150 mg/kg dosage. These dose-dependent changes in rectal temperature were statistically significant ($p<0.001$). The dose × background illumination interaction was not statistically significant ($p>0.42$). These data are summarized in Table 1.

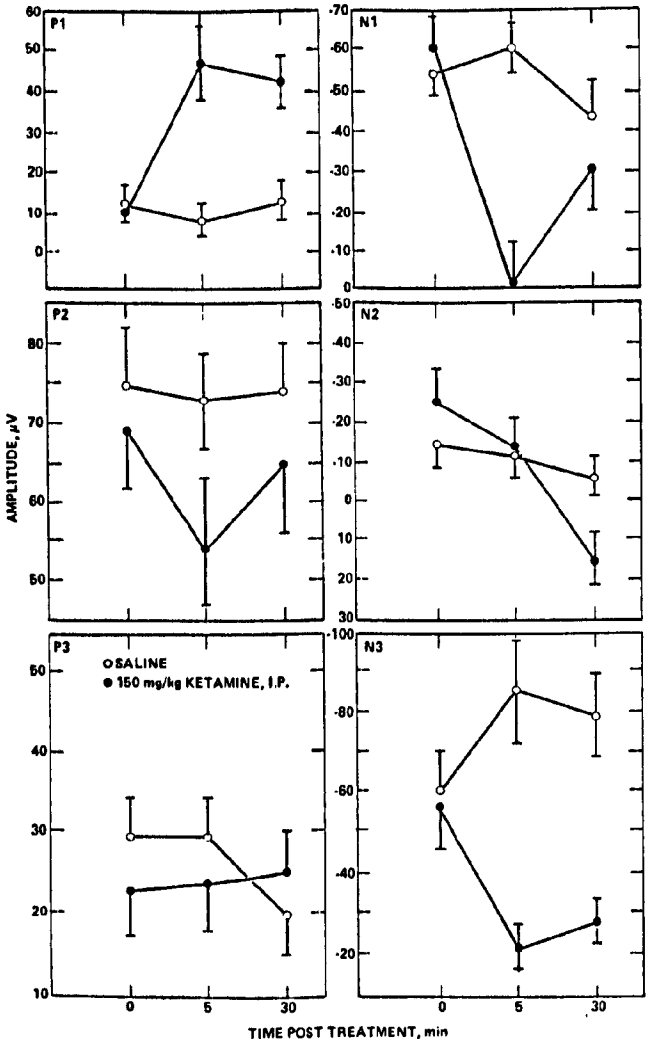

FIG. 4. Mean (±sem) for FEP peak amplitudes obtained immediately before, 5 min after, and 30 min after ketamine (150 mg/kg IP) or saline administration.

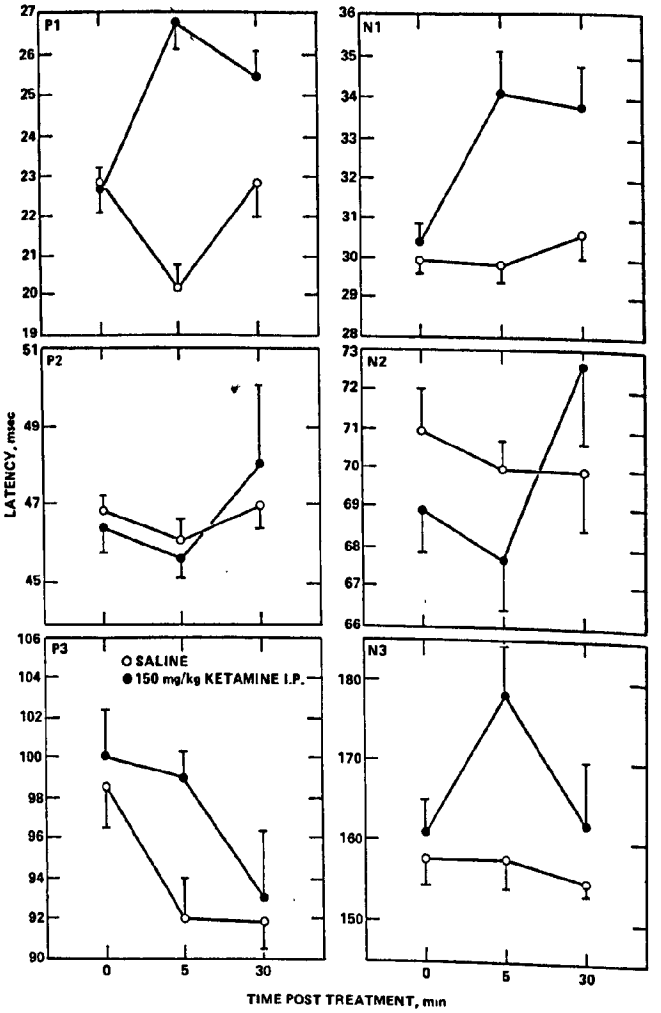

FIG. 5. Mean (±sem) for FEP peak latencies obtained immediately before, 5 min after, and 30 min after ketamine (150 mg/kg IP) or saline administration.

Experiment Two: Time Course of Ketamine Effects

The effects of 150 mg/kg ketamine (IP) on FEPs obtained 0, 5, and 30 min postinjection were compared to FEPs from saline-treated animals tested at the same postinjection times. Both peak latencies and peak amplitudes were significantly altered by ketamine administration

Peak P1 amplitude was increased by ketamine administration both 5 min and 30 min postinjection. The effects of time and dose on peak amplitudes are shown in Fig. 4. Peak N1 amplitude was decreased 5 min and 30 min postinjection by ketamine, but the effect was larger at the 5 min recording time. No effects of dose, time, or any interactions were found on peak P2 or peak P3 amplitude. Peak N2 amplitude decreased significantly during successive recording periods, but no effects of ketamine or dosage × time interactions were observed. Peak N3 amplitude was significantly decreased at 5 min and 30 min postinjection in ketamine-treated animals, but was significantly increased at 5 min and 30 min in saline-treated animals.

Ketamine administration significantly affected peak latencies as well. The effects of dosage and time on peak latencies are depicted in Fig. 5. Peak P1 latency was significantly increased 5 min and 30 min following ketamine injection in contrast to the peak P1 latencies recorded from saline-treated animals which were decreased at the 5 min recording period, and unchanged at the 30 min recording period. Peak N1 latency was significantly increased by ketamine treatment compared to saline-treated animals 5 and 30 postinjection. Peak P2, N2 and P3 latencies were not affected by ketamine treatment. Peak N3 latency was significantly increased 5 min, but not 30 min postinjection in ketamine-treated animals, while peak N3 latencies from saline-treated animals were unchanged.

When measured 1 hour after administration of 150 mg/kg ketamine, rectal temperatures were significantly elevated with respect to controls (39.3±0.3°C vs. 38.0±0.1°C).

DISCUSSION

Peak latencies and peak amplitudes of rat FEPs were altered by ketamine administration. Effects were observed following subanesthetic (37, 75 mg/kg) and anesthetic (150 mg/kg) dosages of ketamine and were qualitatively different

from the effects of other general anesthetics [10, 17, 18], which act through different mechanisms. Effects on peaks P1, N1, and N3 occurred within 5 min of intraperitoneal ketamine administration and recovered at different rates. Peak N1 was nearly recovered by 30 min, while peak P1 and N3 amplitudes were still significantly altered 30 min after treatment.

It is not likely that these effects are secondary to the effects of ketamine on body temperature, since the hypothermic effect of ketamine was prevented by testing under warm ambient conditions. Furthermore, the major findings in the present study are the great changes in peak amplitude by ketamine. Changes in body temperature of as much as several degrees celsius have been shown not to produce such changes in amplitude [10,17].

Ketamine, a short-duration, nongaseous anesthetic [32,33], selectively blocks the action of excitatory amino acids on NMDA receptors in vitro at micromolar concentrations [15], and in vivo at subanesthetic dosages [1,22]. This selective antagonism has been demonstrated in spinal cord [3, 20, 22], hippocampus [8], and cortex [14, 26, 27]. NMDA receptors mediate a magnesium sensitive, voltage-dependent depolarization [3,8]. The NMDA receptor is linked to a cation channel, which under normal conditions is blocked by magnesuim ions. When the neuron membrane is depolarized the magnesium ion leaves the channel, and if the NMDA receptor is stimulated, the membrane depolarizes further. Ketamine blocks this response in magnesium free media and when the neuron is depolarized [31]. This antagonism is noncompetitive and ketamine may interact with a separate binding site [23]. The time-course of the effects reported here is similar to the time-course of anesthesia/analgesia induced by ketamine, which has been postulated to be a result of a blockade of NMDA receptor-mediated depolarization [30]. Ketamine has also been reported to block cholinergic receptors, but not as potently as it antagonizes NMDA actions [22]. Compounds which are more potent and selective for blockade of cholinergic receptors do not elicit the same effects on the FEP as ketamine [19]. In fact, the pattern of amplitude changes reported here is quite different from what has been reported following manipulation of cholinergic, dopaminergic, noradrenergic and serotonergic systems ([11], and Dyer, unpublished data). Ketamine has also been shown to block potassium and sodium channels in myelinated nerve fibers, but only at very high concentrations: 2–4 mM [2].

The effects of ketamine on the amplitudes of peaks P2 and N2 were different in light-tested and dark-tested animals. While these findings raise the possibility that light adaptation may play a role in the regulation of the voltage-dependent cation channel associated with NMDA receptors, the mechanism by which light adaptation affects the response to ketamine cannot be specified based upon these data.

The effects of ketamine on FEPs recorded from the lateral geniculate body [7], superior colliculus [16], and somatosensory cortex [7] have been reported. Hetzler and Berger [16] reported an augmentation of early positive components of superior colliculus evoked potentials after 100 mg/kg IP ketamine, as well as emergence of a new positive spike at lower dosages. Dafny and Rigor [7] observed decreased amplitudes of all components of FEPs recorded from rat lateral geniculate body and no consistent effects on somatosensory cortex. Because we studied FEPs recorded from visual cortex, it is difficult to make comparisons to these studies.

Phencyclidine (PCP), which is also presumed to functionally block NMDA receptors, has been investigated using flash evoked potentials [29]. Because this study measured amplitudes using a peak-to-peak rather than a baseline-to-peak scheme, and because no waveforms from treated rats are shown, it is not possible to compare the amplitude effects with the present study. While the reported increases in peak latency are consistent with the present findings, the amplitude effects are the most striking and important feature of the ketamine data presented here. It is therefore not yet possible to compare the effects of these apparently similar compounds on FEPs.

Ketamine has been described as a drug with both convulsant and anticonvulsant properties [25]. Some authors have maintained that the photic afterdischarge which often follows the FEP may provide a useful indicator of whether a drug is a convulsant or anticonvulsant. Convulsants are presumed to increase amplitude of this response, while anticonvulsants are presumed to decrease their amplitude [27]. To the extent that FEP peak N3 reflects the first wave of the photic afterdischarge, the present data support the notion that ketamine's effects are better classified as anticonvulsant than convulsant.

Ketamine administration produced profound alterations in rat cortical FEPs. Available evidence indicates that functional blockade of NMDA receptor-mediated depolarization contributes to the analgesic [1,22], anesthetic [1,22], and psychotomimetic [27] ketamine effects. Further studies need to be performed using other NMDA receptor antagonists, but the reported findings support the hypothesis that excitatory amino acid stimulation of NMDA receptors plays a role in the generation of rat visual cortex flash evoked potentials.

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